

OBJECTIVES

- Separate plant pigments using chromatography
- Calculate R_f values for four different plant pigments
- Measure the rate of photosynthesis using a spectrophotometer

MATERIALS

MATERIALS NEEDED PER GROUP

- 1 Vial
- 1 Chromatography paper strip
- 4 Cuvettes
- 15 Pipets
- Chromatography solvent, 10 ml
- Quarter
- Spinach
- Indophenol solution, 3 ml
- Phosphate buffer, 4 ml
- Prepared boiled chloroplast
- Prepared unboiled chloroplast
- Parafilm
- Test tube rack
- Spectrophotometer
- Floodlight, 100 W
- Aluminum foil



DID YOU KNOW?

A typical plant cell may contain as many as fifty chloroplasts.

PROCEDURE

PART A: CHROMATOGRAPHY OF PLANT PIGMENTS



Protective gloves, goggles, and an apron should be worn throughout this activity.



When working with the chromatography solvent, use a chemical hood or proper ventilation. Refer to the enclosed MSDS for disposal instructions for the solvent.

1. Obtain a chromatography vial from your teacher and label it with your initials using a permanent marker or wax pencil.

Figure 1

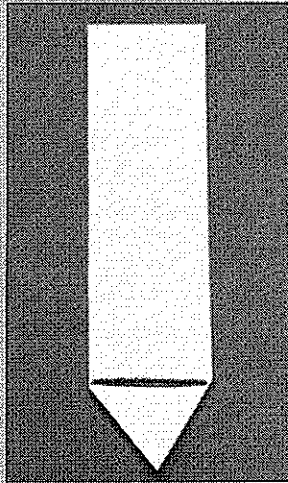


Figure 2

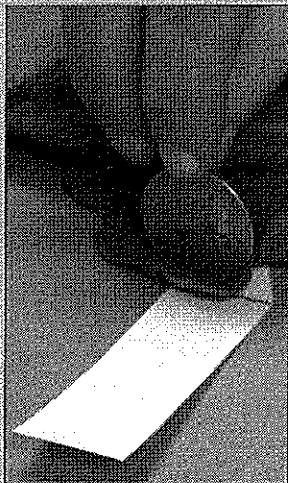
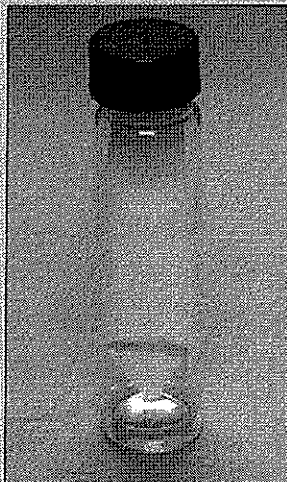


Figure 3



Step 2 should be performed under a chemical fume hood or with proper ventilation.

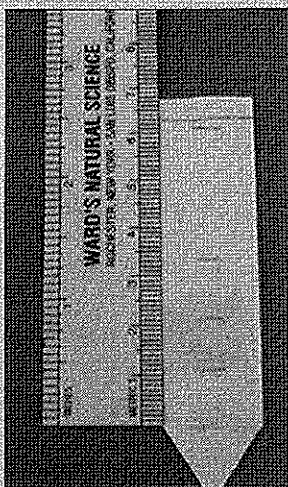
2. Go to a fume hood or a well ventilated area and remove the cap from a chromatography vial. Using a disposable pipet, add 1 ml of chromatography solvent to the vial. Replace the cap and allow the chamber to sit undisturbed until needed in Step 7. This will ensure that the atmosphere within the vial is saturated with solvent vapors (equilibration).
3. Obtain a chromatography strip from your instructor. Be sure to handle the chromatography strip by the edges. Do not touch the surface of the strip. The oils from your fingers can interfere with the chromatogram.
4. Measure 1.5 cm from one end of the chromatography strip and draw a pencil line across the width of the strip.
5. Use a pair of scissors to cut two small pieces below the pencil line to form a pointed end (Figure 1). The pointed end will be referred to as the bottom end of the chromatogram.
6. Obtain a fresh piece of spinach and place it over the line on the chromatography strip. Rub the ribbed edge of a coin (dime or quarter) over the spinach leaf to extract the pigments. Repeat 5 to 10 times with different portions of the spinach leaf, making sure you are rubbing the coin over the pencil line (Figure 2).



Steps 7-11 should be performed under a chemical fume hood or with proper ventilation.

7. Remove the cap from your chamber and carefully place the chromatography strip into the vial so that the pointed end is barely immersed in the solvent. Make sure not to immerse the pigments in the solvent (Figure 3).
8. Cap the vial and leave it undisturbed. Observe as the solvent is drawn up the chromatography strip by capillary action. You will be able to see the plant pigments separating along the strip. Notice the different colors that you see during this process.
9. When the solvent reaches approximately 1 cm from the top of the strip, remove the cap from the vial. Using forceps, remove the strip from the vial. This is a chromatogram.

Figure 4



10. Immediately mark the location of the solvent front. The solvent will evaporate quickly.
11. In Table 2 in the Analysis section, list the pigment colors that you observe. Once the strip has dried, mark the middle of each pigment band on your chromatography strip with a pencil.
12. Using a metric ruler, measure the distance from the original pencil line with the spinach extract to the solvent front and each mark you have made for each pigment band (Figure 4). Record these distances in millimeters in Table 1 in the Analysis section.
13. Calculate the R_f value for each pigment on your chromatogram using the following formula and record your answers in Table 1.

$$R_f = \frac{\text{Distance pigment traveled}}{\text{Distance solvent traveled}}$$

14. Follow your teacher's instructions for proper disposal of all materials.

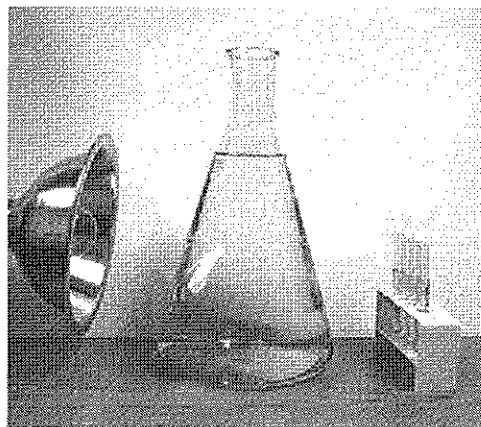


Refer to the MSDS for proper disposal of chromatography solvent.

Part B: Photosynthesis / The Light Reaction

1. Turn on the spectrophotometer to warm it up. Refer to the instruction manual for your spectrophotometer to determine how long it will take to warm up. Adjust the wavelength control knob to 605 nm.
2. Set up an incubation area with a floodlight, a flask of water for a heat sink, and a test tube rack or similar sample holder (Figure 5).

Figure 5





DID YOU KNOW?

Many scientists believe that chloroplasts and mitochondria have evolved from prokaryotic "trespassers" that invaded other, larger cells, a speculation known as the endosymbiotic theory.

3. With a glass-marking pen, label four cuvettes at the very top rim 1, 2, 3, and 4. Wipe down the outside of each cuvette with lens paper to remove any fingerprints and oils.
4. Wrap the outside of cuvette 2 with foil and make a foil cap for the top to keep the chloroplast solution in complete darkness. It will be used as the experiment control.
5. Add the following:

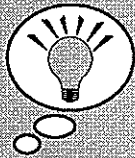
	Cuvette			
	1	2	3	4
Phosphate Buffer	1 ml	1 ml	1 ml	1 ml
Distilled Water	4 ml	3 ml	3 ml	3 ml
DPIP	—	1 ml	1 ml	1 ml

6. Zero the spectrophotometer by adjusting the amplifier control knob until the meter reads 0% transmittance.
7. Obtain 2 ml of boiled chloroplast and 2 ml of unboiled chloroplast. Transfer three drops of unboiled chloroplasts to cuvette 1. Cover the top of the cuvette with parafilm and invert to mix.
8. Place cuvette 1 in the sample holder of the spectrophotometer. Be sure the cuvette is wiped clean and is inserted into the sample holder in the same direction every time to ensure consistent readings. Adjust the light control knob until the meter reads 100% transmittance.



Cuvette 1 will be used to recalibrate the spectrophotometer between readings.

9. Transfer three drops of unboiled chloroplasts into cuvette 2 with a pipette. Immediately cover the cuvette with parafilm and invert to mix.
10. Remove the foil sleeve and foil top, and place the cuvette in the sample holder. Read the percent transmittance and record it as Time 0 in Table 2 in the Analysis section of the lab.
11. Place the cuvette back into its foil sleeve and cover it with the foil top. Place the cuvette in the test tube rack between percent transmittance readings.
12. Repeat the readings at 5, 10, and 15 minutes. Be sure to cover and mix the cuvette before each reading. Read the percent transmittance and record the data in Table 2.



DID YOU KNOW?

The cartoon character "Popeye" attributes his amazing strength to a daily diet of spinach. In fact, spinach is an excellent source of both vitamin A and folic acid, as well as iron and potassium.



Be sure to use cuvette 1 to check and recalibrate the spectrophotometer between each reading to ensure consistent results.

13. Transfer three drops of the unboiled chloroplasts into cuvette 3. Immediately cover the cuvette with parafilm and invert to mix.
14. Place the cuvette in the sample holder. Read the percent transmittance and record it as Time 0 in Table 2.
15. Place the cuvette in the test tube rack between percent transmittance readings.
16. Repeat the readings at 5, 10, and 15 minutes. Be sure to cover and mix the cuvette before each reading. Read the percent transmittance and record the data in Table 2.



Be sure to use cuvette 1 to check and recalibrate the spectrophotometer between each reading to ensure consistent results.

17. Transfer three drops of the boiled chloroplasts into cuvette 4. Immediately cover the cuvette with parafilm and invert to mix.
18. Place the cuvette in the sample holder. Read the percent transmittance and record it as Time 0 in Table 2.
19. Place the cuvette in the test tube rack between percent transmittance readings.
20. Repeat the readings at 5, 10, and 15 minutes. Be sure to cover and mix the cuvette before each reading. Read the percent transmittance and record the data in Table 2.



Be sure to use cuvette 1 to check and recalibrate the spectrophotometer between each reading to ensure consistent results.

ANALYSIS

Table 1
Chromatography of Plant Pigments

Band Number	Pigment	Migration Distance (mm)	R _f Value
1 (top)			
2			
3			
4			
—			

Table 2
% Transmittance

Cuvette	Time			
	0 min.	5 min.	10 min.	15 min.
2 (Dark)				
3 (Unboiled)				
4 (Boiled)				

WARD'S
AP Biology Lab #4
Plant Pigments and Photosynthesis
Lab Activity

Name: _____
Group: _____
Date: _____

ASSESSMENT

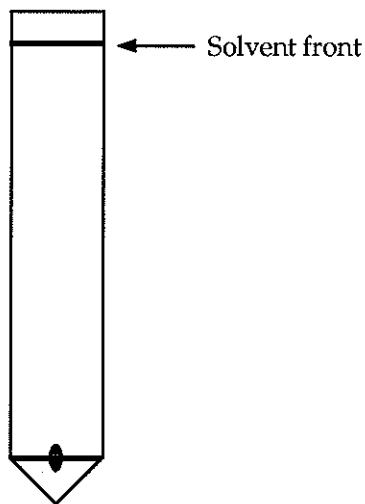
1. Which pigment migrated the farthest? Why?
2. During summer, leaves are generally bright green. What would you hypothesize that this indicates about the role of green light wavelengths, chlorophyll, and the photosynthetic process?
3. Design an experiment to test your hypothesis from the question above. Describe your experiment or draw a picture of your experimental setup. If you draw your setup, be sure to label each component and its purpose.
4. Why do leaves change color in autumn?
5. What is the function of the chlorophylls in photosynthesis?

6. What are the accessory pigments and what are their functions?

7. In your experiment you used paper chromatography to separate various pigment molecules. There are several other chromatographic techniques employed to separate a variety of molecules. Research another form of chromatography and describe it below.

8. What does the R_f value represent? If you were to perform your experiment on a chromatography strip that was twice the length of the one you used, would your R_f values still be the same?

9. Shown below is a strip of chromatography paper and a list of five molecules and their various R_f values. Assuming the solvent front traveled 54 mm, place each molecule where it would be found on the finished chromatogram.



Molecule	R_f value
N,N-fictionol	.41
cis-2,4-pretendium	.20
dl-made-upelene	.72
d(+)-tetra-imaginase	.91
polysynthetic acid	.78

10. What is the absorption spectrum?

11. In what way is the spectrophotometer used to measure the rate of photosynthesis?

12. Below is graphic representing the steps in the process of photosynthesis. Place the components from the list into their proper place on the graphic.

